

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

Date: March 16, 2006

MEMORANDUM

SUBJECT:

1,2,4-Triazole: Response to the U.S. Triazole Task Force waiver request

for the aerobic aquatic (162-4) and anaerobic aquatic (162-3) metabolism

studies.

PC Code:

600074

DP Number:

320683

FROM:

Iwona Maher, Chemist, ERB 1 Hell School 3/16/06

James Hetrick, Ph.D., Senior Physical Scientist, ERB1 Janua at High id 3/16/06

Nader Elkassabany, Ph.D., Acting Branch Chief, ERB1 Madel 82 keases 3/16/06

TO:

Tamue Gibson/Cynthia Giles-Parker, FB

Registration Division (7505C)

The aerobic aquatic and anaerobic aquatic metabolism studies should be reserved at this time. EFED is recommending reserving the studies because there may be a need to refine the 1,2,4-triazole drinking water assessment in the future. Refinement of the drinking water assessment may be required for new registrations of triazole-derivative fungicides with triazole production greater than 30.7% of applied parent and/or a maximum annual application rate greater than 10.38 lbs a.i./acre. Under these conditions, the 1,2,4-triazole concentration will be higher than the Tier II drinking water assessment (D320682; Feb 28, 2006).

Text Searchable File

Data Evaluation Report on the anaerobic biotransformation of 1,2,4-triazole (CGA 71019) in soil

PMRA Submission Number {.....}

EPA MRID Number 45930701

Data Requirement:

PMRA Data Code:

EPA DP Barcode:

D320683

OECD Data Point:

EPA Guideline:

162-2

Test material:

Common name:

1,2,4-Triazole.

Chemical name:

IUPAC name:

4H-[1,2,4]Triazole.

CAS name:

Not reported.

CAS No:

288-88-0.

Synonyms:

CGA 71019; Pyrrodiazole.

SMILES string:

n1cnnc1 (ISIS v2.3/Universal SMILES).

n1ncnc1 (EPI Suite, v3.12).

Primary Reviewer: Lynne Binari

Signature: Lynne Reneur

Cambridge Environmental Inc

Secondary Reviewer: Kathleen Ferguson

Cambridge Environmental Inc

Date: 2/23/06

Signature: Kathleen Juguson
Date: 2/23/06

QC/QA Manager: Joan Gaidos

Cambridge Environmental Inc

Signature:

Date: 2/23/06

Final Reviewer: Iwona Maher

EPA Reviewer

Company Code:

Active Code:

Use Site Category:

EPA PC Code:

600074

CITATION: Mamouni, A. 2003. [14C]-CGA 71019: anaerobic soil degradation. Unpublished study performed by RCC, Ltd., Itingen, Switzerland; sponsored and submitted by Syngenta Crop Protection, Inc., Greensboro, North Carolina. RCC Project and Study No.: 798660. Experimental start date January 25, 2001, and termination date July 17, 2001 (p. 12). Final report issued January 28, 2003.

PMRA Submission Number {.....}

EPA MRID Number 45930701

EXECUTIVE SUMMARY

The biotransformation of [3.5-14C]-4H-[1,2,4]triazole (CGA 71019) was studied in silt loam soil (pH 7.31, organic carbon 2.11%) from Switzerland for 122 days in darkness at 20 ± 2 °C under anaerobic conditions (flooding, nitrogen atmosphere) following 4 days of aerobic incubation. Soil moisture content prior to flooding was 40% of maximum water holding capacity. [14C]Triazole was applied at a rate of 0.061 mg a.i./kg soil dry wt. (equivalent to 0.046 kg a.i./ha). This study was conducted in accordance with Council Directive 91/414/EEC, Annex II, 7.1.1.2 rate of degradation, 7.1.1.2.1 aerobic degradation as amended by Commission Directive 95/36/EC (1995) and OECD Guidelines for the Testing of Chemicals, Draft Guideline 307 Aerobic and Anaerobic Transformation in Soil (2000), and in compliance with the Swiss Good Laboratory Practice Ordinance RS 813.016.5 (2000) based on OECD Principles of GLP (1997). The test system consisted of 1-L glass metabolism flasks with attached traps for the collection of CO₂ (2N NaOH) and volatile organics (ethylene glycol). Treated soil samples were taken for analysis after 0, 3 and 4 days of aerobic incubation, then after conversion to anaerobic conditions, water-soil systems were taken at 9, 11, 18, 31, 64 and 126 days posttreatment (5, 7, 14, 27, 60 and 122 days post-flooding, respectively). Duplicate samples were taken at all intervals, except 3 and 31 days posttreatment when single treated samples were collected. Water, when present, was separated from the soil via pipette, then concentrated via rotary evaporation and analyzed directly. Soil samples were extracted three to four times with acetonitrile; water (8:2, v:v), followed by once with water (0- to 4-day posttreatment samples only), and finally reflux (Soxhlet) extracted for ca. 4 hours with acetonitrile:water (9:1, v:v). Soil extracts were combined and concentrated using rotary evaporation. Soil extracts, extracted soil, water layers and trapping solutions were analyzed for total radioactivity using LSC. Soil extracts and water layer samples were analyzed for [14C]triazole and its transformation products using normal-phase, one-and twodimensional TLC. Identifications were made via co-chromatography against the following unlabeled reference standards: [1,2,4]triazol-1-yl-acetic acid (CGA 142856), 2-amino-3-[1,2,4]triazol-1-ylpropionic acid (CGA 131013) and 4H-[1,2,4]triazol-3-ol (NOA 457654). Identifications were not confirmed using a second method.

The test conditions outlined in the study appear to have been maintained throughout the 126-day incubation. After flooding the soil at 4 days posttreatment, the conditions became increasingly anaerobic with measured redox potentials of +185 to +206 mV and +141 to +145 mV in the water and soil, respectively, at 5 days post-flooding decreasing to -249 to -198 mV and -277 to -259 mV, respectively, at study termination. In the water layers, dissolved oxygen decreased from 1.00-3.50 mg/L at 5 days post-flooding to ≤0.30 mg/L at 27-122 days post-flooding, while pH levels gradually increased from 7.61-7.81 at 5 days to 8.39-8.70 at study termination.

Recoveries of radiolabeled material averaged $96.3 \pm 0.9\%$ of the applied (range 93.9-97.3%), with no pattern of decline during the 126-day study. After flooding, [14 C]residues partitioned between the soil and the water layer with a consistent distribution ratio (water:soil) of 1:2.

During aerobic incubation, [¹⁴C]triazole in the soil decreased from a mean (individual replicate results were not reported) 89.9% of the applied at day 0 to 56.5% at 4 days, then post-flooding,

PMRA Submission Number {.....}

EPA MRID Number 45930701

decreased to 21.1% in the total system at 126 days (122 days post-flooding). The dissipation rate of [\frac{14}{C}]\text{triazole} appeared to slow with the conversion to anaerobic conditions; however, there were not a sufficient number of sampling intervals during the aerobic incubation to allow for an accurate comparison. In the water layer, [\frac{14}{C}]\text{triazole} gradually decreased from 20.0-22.4% at 9-11 days posttreatment (5-7 days post-flooding) to 9.0% at 64 days (60 days post-flooding) and was 4.6% at study termination. In the soil, [\frac{14}{C}]\text{triazole} decreased from 56.5% at 4 days (day 0 prior to flooding) to 27.3% at 64 days and was 16.5% at study termination. Based on first order linear regression and nonlinear analyses, half-life values of [\frac{14}{C}]\text{triazole} post-flooding were 46-55, 72-88 and 82-84 days in the water, soil and total system, respectively, with observed DT50 values of 27-60 days post-flooding in the water layer and 60-122 days in the soil and total system.

One major transformation product,

[1,2,4]triazol-1-yl-acetic acid (CGA 142856),

and one minor product,

2-amino-3-[1,2,4]triazol-1-yl-propionic acid (CGA 131013),

were tentatively identified (identifications were not confirmed). **CGA 142856** was detected in the soil at a mean 15.2% of the applied at 4 days posttreatment prior to flooding, then was 18.3-18.5% in the total system (10.6-11.5% in water layer, 6.8-7.9% in soil) at 9-11 days posttreatment (5-7 days post-flood) and increased to 50.3% (30.0% in water, 20.3% in soil) at study termination. **CGA 131013** was detected at maximum mean levels of 3.4% in the soil and total system and was \leq 0.1% in the water layer. Unidentified radioactivity consisted of [14 C]residues remaining at the plate origin plus four unidentified [14 C]compounds, each detected at maximum mean values of 0.4-1.0%, 0.4-3.1% and 0.9-3.1% in the water, soil and total system, respectively. Extractable soil [14 C]residues decreased from 90.5-91.9% of the applied at day 0 posttreatment to 40.2-42.2% at study termination. Nonextractable [14 C]residues increased from 5.0-5.6% of the applied at day 0 to 20.9-21.9% at 64 days (60 days post-flood) and were 15.5-17.2% at study termination. At study termination, volatilized 14 CO₂ and volatile [14 C]organic compounds totaled 1.3-3.9% and <0.2% of the applied, respectively.

The study author did not provide a transformation pathway. Based on the transformation products detected, acetylation of triazole largely yielded [1.2.3]triazol-1-yl-acetic acid (CGA 142856), with amination then yielding minor amounts of 2-amino-3-[1,2,4]triazol-1-yl-propionic acid (CGA 131013).

PMRA Submission Number {.....}

EPA MRID Number 45930701

Results Synopsis:

Test system used: Silt loam soil flooded with distilled water.

Half-life/DT50 values reported as post-flood.

Linear half-life in water: 54.8 days ($r^2 = 0.9635$). Linear half-life in soil: 88.4 days ($r^2 = 0.8401$). Linear half-life in the total system: 83.6 days ($r^2 = 0.9724$)

Non-linear half-life in water: 45.9 days ($r^2 = 0.9233$). Non-linear half-life in soil: 72.2 days ($r^2 = 0.7218$). Non-linear half-life in total system: 81.5 days ($r^2 = 0.9479$).

Observed DT50 in water: 27-60 days.
Observed DT50 in soil: 60-122 days.
Observed DT50 in total system: 60-122 days.

Major transformation products: [1,2,4]Triazol-1-yl-acetic acid (CGA 142856, M1; maximum

mean 30.0%, 20.3% and 50.3% of applied in water, soil and

total system, respectively).

Minor transformation products: 2-Amino-3-[1,2,4]triazol-1-yl-propionic acid (CGA 131013,

M2; maximum mean 0.1% in water and 3.4% in soil and total

system).

CO₂ (maximum 3.9% of applied). Volatile organics (<0.2% of applied).

Study Acceptability: This study is classified as **acceptable**. It is scientifically valid. No significant deviations from the objectives of Subdivision N guidelines were noted.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted in accordance with Council Directive

91/414/EEC, Annex II, 7.1.1.2 rate of degradation, 7.1.1.2.1 aerobic

degradation as amended by Commission Directive 95/36/EC (1995); and OECD Guidelines for the Testing of Chemicals, Draft Guideline 307 Aerobic and Anaerobic Transformation in Soil (2000, p. 13). No significant deviations from the objectives of

Subdivision N guidelines were noted.

COMPLIANCE: This study was conducted in compliance with the Swiss Good

Laboratory Practice Ordinance RS 813.016.5 (2000) based on OECD Principles of GLP (1997, p. 3). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were

PMRA Submission Number {.....}

EPA MRID Number 45930701

provided (pp. 2-3, 5-6, 8). A Certificate of Authenticity was not provided.

A. MATERIALS:

1. Test Material

[3,5-¹⁴C]-4H-[1,2,4]triazole (CGA 71019; p. 16).

Chemical Structure:

See DER Attachment 1.

Description:

Technical; physical state not reported (p. 17).

Purity: Radiochemical purity:

>97% (pp. 17, 25; Figure 6, p. 39).

Lot/Batch No.

CFQ12197 (Nycomed Amersham).

RCC No.: 111069/A (p. 16).

Analytical purity:

Not reported.

Specific activity:

29.76 MBq/mg (p. 17).

Location of the radiolabel:

At 3-C and 5-C of the triazole ring (p. 16).

Storage conditions of

test chemicals:

Radiolabeled test substance was stored frozen (ca. -

20°C) in darkness (p. 17). Unlabeled test substance

was stored at $<+10^{\circ}$ C in the dark (p. 16).

Physico-chemical properties of triazole (CGA 71019):

Parameter	Value	Comment
Molecular weight	69.07 g/mol	
Molecular formula	$C_2H_3N_3$	
Water Solubility	Not reported.	
Vapor Pressure/Volatility	Not reported.	
UV Absorption	Not reported.	
Pka	Not reported.	
K _{ow} /log K _{ow}	Not reported.	
Stability of compound at room temperature	Not reported.	

Data obtained from p. 16 of the study report.

PMRA Submission Number {.....} EPA MRID Number 45930701

2. Soil Characteristics

Table 1: Description of soil collection and storage.

Description	Details
Geographic location	Field located in Les Barges, VS/Switzerland.
Pesticide use history at the collection site	Not reported.
Collection date	October 10, 2000.
Collection procedures	Not reported.
Sampling depth (cm)	0- to 20-cm layer.
Storage conditions	Maintained under plant cover in a greenhouse until soil preparation (see below) <i>ca.</i> 2 weeks prior to use.
Storage length	ca. 107 days based on experimental start date of January 25, 2001.
Soil preparation	The soil was 2-mm sieved and stored at ca. 20°C in an open bag ca. 2 weeks prior to use. During this period, the soil was frequently mixed and finger-crumbled to avoid surface drying. Just prior to use, the soil moisture was adjusted to 40% of maximum water holding capacity.

Data obtained from pp. 12, 17 of the study report.

PMRA Submission Number {.....} EPA MRID Number 45930701

Table 2: Properties of the soil.

Property		Details
Soil texture		Silt loam.
% Sand (0.05-2.0 n	nm)	35.88
% Silt (0.002-0.05	mm)	52.28
% Clay (<0.002 mr	n)	11.83
pH (in KCl)		7.31
Organic carbon (%)		2.11
CEC(meq/100g)		13.86
Moisture at 1/3 atm (%)	Not reported.
	MWC 1	68.29
Moisture (%)	40% MWC	27.32
	Field capacity	47.86
Bulk density (g/cm³)		Not reported.
Microbial biomass (mg C/100 g dry wt. soil) ²		29.8
Soil taxonomic classification		Not reported.
Sol mapping unit (for	r EPA)	Not reported.

¹ Maximum water holding capacity.

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: No primary experiments were reported.

2. Experimental conditions:

Table 3: Experimental design.

Parameter		Details	
Duration of the test		126 days; 4 days of aerobic incubation followed by 122 days of anaerobic incubation.	
Soil condition: (Air dried/fresh)		Fresh.	
Soil (g/replicate)		100 g dry wt.	
Application rates (mg a.i./kg and equivalent kg	Nominal:	0.06 mg a.i./kg (0.05 kg a.i./ha).	
a.i./ha)	Actual:	0.061 mg a.i./kg (0.046 kg a.i./ha).	

² Microbial biomass determined in soil treated with 310 µL acetone:water (1:1, v:v, test solution vehicle, p. 19). Data obtained from p. 19; Table 1, p. 28 of the study report.

PMRA Submission Number {.....}

EPA MRID Number 45930701

Parameter		Details			
Control conditions, if used		Sterile controls were not prepared.			
No. of	Controls, if used	Sterile controls were not prepared.			
Replications	Treatment	Duplicate treated soil samples at e. for 3 and 31 days posttreatment wl			
Test	Type/material/volume	1-L glass metabolism flasks (i.d. continuous flow-through system.	a. 10.6 cm) attached to a		
apparatus	Details of traps for CO ₂ and organic volatiles, if any	2N NaOH to trap CO ₂ ; one trap, 5 Ethylene glycol to trap organic vo	0 mL. latiles; one trap, 50 mL.		
If no traps we closed/open?	re used, is the system	Volatiles traps were used.			
Identity and c	oncentration of co-solvent	Acetone; final concentration 0.129 of acetone:water (1:1, v:v) test sol			
	Volume of the test solution used/treatment:	310 μL.	310 μL.		
	Application method (eg: mixed/not mixed):	Applied dropwise via Hamilton syringe to soil surface, ther incorporated by mixing.			
	Is the co-solvent evaporated?	Not reported.			
	n of the test material adsorbing f the test apparatus?	Not indicated.			
Microbial bio	mass/microbial population of	Initial	Final		
the control (u	nits)	Sterile controls were not prepared.			
Microbial bio	mass/microbial population of	Initial	Final		
the treated so	il (units)	Treated soil samples were not analyzed for biomass.			
Temperature (°C):		20 ± 2°C.			
Experimental	Continuous darkness (Yes/No):	Yes.			
conditions:	Moisture content: Moisture maintenance method:	40% of maximum water holding capacity. Not required as treated soils were flooded after 4 days of aerobic incubation.			
Other details,	if any	None.			

Data obtained from pp. 17-19; Figure 1, p. 34 of the study report.

3. Aerobic/anaerobic conditions: Following treatment, the test soils were incubated aerobically (humidified air, continuous flow at *ca.* 30 mL/minute) for 4 days, then converted to anaerobic conditions by flooding with *ca.* 200 mL of degassed (ultrasonic bath, nitrogen purge), distilled water plus conversion to nitrogen atmosphere (10 minute purge at 30 mL/minute, four times daily; p. 18). The water layer depth was 1-2 cm above soil surface (p. 18). After 5 days of anaerobic incubation (first anaerobic sampling interval, 9 days posttreatment), redox potentials and dissolved oxygen in the

PMRA Submission Number {.....}

EPA MRID Number 45930701

water layers were +185 to +206 mV and 1.00-3.50 mg/L, respectively, with redox potentials in the soil of +141 to +145 mV (Table 3, p. 30).

4. Supplementary experiments: None reported.

5. Sampling:

Table 4: Sampling details.

Criteria	Details
Sampling intervals (posttreatment)	0, 3, 4, 9, 11, 18, 31, 64 and 126 days.
Sampling method	Duplicate treated soil samples at all intervals except 3 and 31 days posttreatment when a single treated sample was taken.
Method of collection of CO ₂ and organic volatile compounds	Trapping solutions were collected and replaced at each sampling interval plus at 22 and 109 days posttreatment (18 and 105 days of anaerobic incubation).
Sampling intervals/times for: Sterility check, if sterile controls are used: Moisture content: Redox potential, dissolved oxygen and pH in the water layer and redox potential in the soil:	Sterile controls were not used. Not reported. Measured at each sampling interval.
Sample storage before analysis	Not reported.
Other observation, if any (eg: precipitation, color change etc.)	None reported.

Data obtained from pp. 19-20; Table 4, p. 31 of the study report.

C. ANALYTICAL METHODS:

Separation of the water and soil: The water layer, when present, was drawn off via glass pipette, then duplicate aliquots (up to 1 mL) were analyzed for total radioactivity by LSC (pp. 20-22).

Extraction/clean up/concentration methods for water and soil samples: The water layer was concentrated via rotary evaporation (30°C) under reduced pressure (p. 20; Figure 2, p. 35).

Soil samples were extracted three to four times with acetonitrile:water (8:2, v:v), followed by once with water (0- to 4-day posttreatment samples only) and finally reflux (Soxhlet) extracted for *ca*. 4 hours with acetonitrile:water (9:1, v:v); extraction solvent volumes were *ca*. 1 mL/g soil (p. 20; Figure 2, p. 35). The acetonitrile:water (8:2, v:v) and water extractions were done using a shaker (mechanism not specified) at 200-250 strokes/minute for *ca*. 30 minutes/extraction; separation of soil and extract was not described. Duplicate aliquots (up to 1 mL) of each extract were analyzed for total radioactivity by LSC, then the extracts were combined and concentrated using rotary evaporation (30-35°C; pp. 20, 22).

PMRA Submission Number {.....}

EPA MRID Number 45930701

Determination of non-extractable residues: Extracted soil was air-dried and homogenized (method not described), then triplicate aliquots (*ca.* 1.0 g x 3) were analyzed for total radioactivity by LSC following combustion (pp. 20-21).

Determination of volatile residues: Aliquots (up to 1 mL) of the trapping solutions were diluted with water (3-4 mL), then combined with scintillation fluid and analyzed for total radioactivity by LSC (pp. 20, 22).

Total ¹⁴C **measurement:** Total ¹⁴C residues were determined by summing the concentrations of residues measured in the water layers (when present), soil extracts, extracted soil and volatile trapping solutions (Table 4, p. 31).

Derivatization method, if used: None was reported.

Identification and quantification of parent compound: Water and soil extract samples were analyzed using either one-dimensional TLC on normal-phase plates (silica gel 60 F254, 0.25 mm) developed with chloroform:methanol:ammonia [either 90:10:1, v:v (SS17); or 80:20:1, v:v (SS18); or 65:28:8, v:v (SS19)] or two-dimensional TLC on normal-phase plates developed with chloroform:methanol:formic acid:water [75:20:4:2, v:v (SS1)] in the first dimension followed by chloroform:methanol:water [70:25:4, v:v (SS2)] in the second dimension (p. 22). Following development, areas of radioactivity were detected and quantified using a BAS 1000 Fujix Phosphor Imager or a Berthold CHROMA Automatic TLC-Linear Analyser (p. 23). Parent [14C]triazole was identified by co-chromatography with unlabeled reference standard which was visualized using iodine vapor saturation (pp. 22-23; Figure 7, p. 40; Figure 13, p. 46).

Identification and quantification of transformation products: Transformation products were separated and quantified using TLC as described for the parent (pp. 22-23; Figures 7-15, pp. 40-48).

Table 5: Reference compounds available for identifying transformation products of triazole.

Applicant code RCC code	Chemical Name	Purity	Lot/Batch No.
CGA 131013 R1 (102411/E)	2-Amino-3-[1,2,4]triazol-1-yl-propionic acid	97 ± 2%	F-3225
CGA 142856 R2 (102411/F)	[1,2,4]Triazol-1-yl-acetic acid	100 ± 2%	RV-1508/8
NOA 457654 R3 (102411/l)	4H-[1,2,4]Triazol-3ol	99 ± 2%	TE-1523

Data obtained from Table 2, p. 29 of the study report.

Identification of radioactivity recovered in the NaOH trapping solutions was reportedly confirmed as ¹⁴CO₂ via barium chloride precipitation; however, supporting quantitative results were not provided (p. 20).

PMRA Submission Number {.....}

EPA MRID Number 45930701

Detection limits (LOD, LOQ) for the parent compound: Limits of detection (LOD) and quantitation (LOQ) for LSC analyses were reported as 0.04% and 0.06% of the applied, respectively, for initial soil extracts, 0.08% and 0.12%, respectively, for reflux extracts, 0.04% and 0.05%, respectively, for soil combustions, and 0.04% and 0.05%, respectively, for NaOH trapping solutions (Appendix B, p. 53).

LOD and LOQ for TLC analysis were reported as 3.10% and 4.64% of the applied, respectively, for parent triazole (Appendix B, p. 55).

Detection limits (LOD, LOQ) for the transformation products: The LOD and LOQ for LSC were the same as those described for the parent compound.

LOD and LOQ for TLC analysis were reported as 3.678% and 5.517%, respectively, for M1, 0.933% and 1.40%, respectively, for M2, 2.483% and 3.725%, respectively, for M3 (origin), 1.097% and 1.645%, respectively, for M4 and 1.134% and 1.70%, respectively, for M5 (Appendix B, p. 55).

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS: After flooding the soil at 4 days posttreatment, the conditions became increasingly anaerobic decreasing from moderately reducing (-50 to +200 mV) at 5 days postflooding (9 days posttreatment), with measured redox potentials of +185 to +206 mV and +141 to +145 mV in the water and soil, respectively, to strongly reducing (-400 to -200 mV) at 122 days postflooding (126 days posttreatment), with redox potentials of -249 to -198 mV and -277 to -259 mV, respectively (Table 3, p. 30). In the water layers, dissolved oxygen decreased from 1.00-3.50 mg/L at 5 days post-flooding to ≤0.30 mg/L at 27-122 days post-flooding, while pH levels gradually increased from 7.61-7.81 at 5 days to 8.39-8.70 at study termination. Microbial biomass was 29.8 mg C/100 g soil at study initiation (p. 25); microbial activity of treated soil samples was not determined at study termination.

B. MATERIAL BALANCE: Recoveries of radiolabeled material averaged (n = 16) $96.3 \pm 0.9\%$ (range 93.9-97.3%) of the applied, with no pattern of decline during the 126-day study (Table 4, p. 31; DER Attachment 2). After flooding, [14 C]residues partitioned between the soil and the water layer with a consistent distribution ratio (water:soil) of 1:2 (DER Attachment 2).

PMRA Submission Number {.....} EPA MRID Number 45930701

Table 6: Biotransformation of $[3,5^{-14}C]$ -triazole (CGA 71019), expressed as percentage of applied radioactivity (mean \pm s.d.¹, n = 2), in Swiss silt loam soil under anaerobic conditions.

		Sampling times (days)								
Comp	ound	Aerobic				Anaerobic				
		0	3	4	9	11	18	31	64	126
	water			2	20.0	22.4	15.0	13.6	9.0	4.6
Triazole	soil	89.9	63.9	56.5	38.6	37.2	32.1	30.2	27.3	16.5
1	system	89.9	63.9	56.5	58.6	59.6	47.2	43.8	36.2	21.1
	water				10.6	11.5	14.6	15.1	20.1	30.0
$M1^3$	soil	n.d.4	7.8	15.2	7.9	6.8	8.7	9.6	10.9	20.3
	system	n.d.	7.8	15.2	18.5	18.3	23.2	24.7	31.0	50.3
	water				n.d.	n.d.	n.d.	n.d.	n.d.	0.1
M2 ⁵	soil	0.5	2.7	3.3	2.9	3.4	2.6	2.5	0.9	1.2
	system	0.5	2.7	3.3	2.9	3.4	2.6	2.5	0.9	1.4
	water				n.d.	n.d.	0.1	n.d.	0.3	1.0
M3 (origin)	soil	0.9	0.5	1.0	1.7	1.7	2.6	3.1	2.3	1.9
	system	0.9	0.5	1.0	1.7	1.7	2.6	3.1	2.5	2.9
	water				n.d.	n.d.	n.d.	0.4	0.3	n.d.
M4	soil	n.d.	0.6	0.4	n.d.	0.2	0.7	0.9	1.1	0.7
	system	n.d.	0.6	0.4	n.d.	0.2	0.7	1.3	1.4	0.7
	water				n.d.	n.d.	n.d.	0.2	0.4	0.1
M5	soil	n.d.	0.8	0.8	0.4	0.6	0.8	1.7	0.5	0.5
	system	n.d.	0.8	0.8	0.4	0.6	0.8	2.0	0.9	0.6
	water				n.d.	n.d.	n.d.	0.4	n.d.	n.d.
M6	soil	n.d.	n.d.	0.3	0.6	0.2	0.2	1.2	0.5	n.d.
	system	n.d.	n.d.	0.3	0.6	0.2	0.2	1.6	0.5	n.d.
	water				n.d.	n.d.	0.4	0.3	0.2	0.9
M7	soil	n.d.	n.d.	n.d.	n.d.	0.1	0.3	0.4	0.4	n.d.
	system	n.d.	n.d.	n.d.	n.d.	0.1	0.6	0.7	0.6	0.9
Extractable s	oil residues	91.2 ± 0.7	76.2	77.6 ± 2.1	52.0 ± 2.0	50.3 ± 0.3	48.0 ± 1.5	49.7	44.0 ± 2.8	41.2 ± 1.0
Nonextractab	le residues	5.3 ± 0.3	17.1	17.5 ± 1.7	12.6 ± 0.5	11.6 ± 0.8	16.8 ± 1.8	16.2	21.4 ± 0.5	16.4 ± 0.8
CO ₂			0.6	1.0 ± 0.3	1.3 ± 0.0	1.2 ± 0.1	0.7 ± 0.3	1.3	1.0 ± 0.2	2.6 ± 1.3
Volatile orga	nics		<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	< 0.1	<0.2
Total recover	у	96.5 ± 0.4	93.9	96.1 ± 0.1	96.5 ± 0.4	96.8 ± 0.3	95.4 ± 0.3	97.2	96.5 ± 0.7	96.8 ± 0.5

PMRA	Subn	nissic	n Number	{}
T TATIZY F	Duon			1

EPA MRID Number 45930701

- 1 Standard deviations could not be determined for parent [¹⁴C]triazole and transformation products results because the study author only provided the mean result and not individual replicate results. Additionally, only a single soil sample was collected for analysis at 3 and 31 days posttreatment.
- 2 Although "n.d." (not detected) was reported for parent [\frac{14}{2}C]triazole and transformation products in the water layer at day 0 post-flooding (4 days posttreatment), all other information provided in the study report indicates that the soil samples taken at day 4 posttreatment were taken prior to flooding (p. 19; Table 4, p. 31).
- 3 M1 tentatively identified as [1,2,4]triazol-1-yl-acetic acid (CGA 142856, p. 26).
- 4 Not detected. Limits of detection were reported as 3.7%, 0.9%, 2.5%, 1.1% and 1.1% of the applied for M1, M2, M3, M4 and M5, respectively (Appendix B, p. 55).
- 5 M2 tentatively identified as 2-amino-3-[1,2,4]triazol-1-yl-propionic acid (p. 26).
- n.d. = not detected.

Data obtained from pp. 19, 26 and Tables 4-5, pp. 31-32 of the study report.

PMRA Submission Number {.....}

EPA MRID Number 45930701

C. TRANSFORMATION OF PARENT COMPOUND: During aerobic incubation, parent [\frac{14}{C}]\triazole in the soil decreased from a mean 89.9% of the applied at day 0 to 56.5% at 4 days, then post-flooding, decreased to 21.1% in the total system at 126 days (122 days post-flooding; Table 5, p. 32; Reviewer's Comment No. 1). In the water layer, [\frac{14}{C}]\triazole gradually decreased from 20.0-22.4% at 9-11 days posttreatment (5-7 days post-flooding) to 9.0% at 64 days (60 days post-flooding) and was 4.6% at 126 days. In the soil, [\frac{14}{C}]\triazole decreased from 56.5% at 4 days (day 0 prior to flooding) to 27.3% at 64 days and was 16.5% at 126 days.

HALF-LIFE/DT50/DT90: Based on first order linear regression analysis (Excel 2000) and using the reported mean results, the linear half-life values of [\frac{14}{C}]triazole post-flooding were 54.8, 88.4 and 83.6 days in the water, soil and total system, respectively. Based on nonlinear analysis (SigmaPlot v 9), the half-life values of [\frac{14}{C}]triazole post-flooding were 45.9, 72.2 and 81.5 days in the water, soil and total system, respectively. Observed DT50 values were 27-60 days post-flooding in the water layer and 60-122 days in the soil and total system. The rate of dissipation appeared to slow with the conversion to anaerobic conditions; however, there were not a sufficient number of sampling intervals during the aerobic incubation to allow for a valid statistical comparison.

Based on nonlinear analysis, the study author calculated a DT50 value of 80.6 days in the total system using MicroCal Origin v 3.5 and all sampling intervals post-flooding (p. 24; Table 6, p. 33; Figure 5, p. 38).

Half-lives/DT50/DT90

Phase	Half-life/DT50 ¹ (days)	First order linear regression equation	r²	DT50 ² (days)	DT90 ² (days)
Water layer (distil	led, degassed)				
Linear/natural log	54.8	y = -0.0127x + 3.0201	0.9635		
Nonlinear/normal	45.9		0.9233		
Observed DT50	27-60				
Silt loam soil					
Linear/natural log	88.4	y = -0.0078x + 3.7333	0.8401		
Nonlinear/normal	72.2		0.7218		
Observed DT50	60-122				
Total system					
Linear/natural log	83.6	y = -0.0083x + 4.0591	0.9724	80.6	268
Nonlinear/normal	81.5		0.9479		
Observed DT50	60-122				

¹ Determined by the primary reviewer using Excel 2000 (linear) and Sigmaplot v 8.0 (nonlinear) and mean sample data obtained from Table 5, p. 32 of the study report (DER Attachment 2).

TRANSFORMATION PRODUCTS: One major transformation product, [1,2,4]triazol-1-yl-acetic acid (CGA 142856, M1), and one minor product, 2-amino-3-[1,2,4]triazol-1-yl-propionic acid (CGA 131013, M2), were identified via normal-phase, one-and two-dimensional TLC against reference standards (pp. 23, 26; Figures 9-15, pp. 42-48).

[1,2,4]Triazol-1-yl-acetic acid (CGA 142856, M1) was detected in the soil at a mean 15.2% of the applied at 4 days posttreatment prior to flooding, then was 18.3-18.5% in the total system (10.6-11.5% in water layer, 6.8-7.9% in soil) at 9-11 days posttreatment (5-7 days post-flood) and increased to 50.3% (30.0% in water, 20.3% in soil) at 126 days (122 days post-flood; Table 5, p. 32).

2-Amino-3-[1,2,4]triazol-1-yl-propionic acid (CGA 131013, M2) was detected at maximum mean levels of 3.4% at 11 days posttreatment (7 days post-flood) in the soil and total system and was detected only once in the water layer at 0.1% at the final interval.

Unidentified radioactivity consisted of [¹⁴C]residues remaining at the plate origin (M3) plus four compounds, M4-M7, each detected at maximum mean values of 0.4-1.0%, 0.4-3.1% and 0.9-3.1% in the water, soil and total system, respectively.

² Determined by the study authors using MicroCal Origin (nonlinear) and mean sample data post-flooding (p. 24; Table 6, p. 33; Figure 5, p. 38).

PMRA Submission Number {.....}

EPA MRID Number 45930701

NONEXTRACTABLE AND EXTRACTABLE RESIDUES: Extractable soil [¹⁴C]residues decreased from 90.5-91.9% of the applied at day 0 posttreatment to 40.2-42.2% at study termination (DER Attachment 2). Nonextractable [¹⁴C]residues increased from 5.0-5.6% of the applied at day 0 to 20.9-21.9% at 64 days (60 days post-flood) and were 15.5-17.2% at study termination (Table 4, p. 31).

VOLATILIZATION: At study termination (126 days posttreatment), volatilized ¹⁴CO₂ and volatile [¹⁴C]organic compounds totaled 1.3-3.9% and <0.2% of the applied, respectively (Table 4, p. 31).

TRANSFORMATION PATHWAY: The study author did not provide a transformation pathway. Based on the transformation products detected, acetylation of triazole yields [1,2,4]triazol-1-yl-acetic acid (CGA 142856), with amination yielding 2-amino-3-[1,2,4]triazol-1-yl-propionic acid (CGA 131013).

Table 7: Chemical names and CAS numbers for the transformation products of triazole. ¹

Applicants Code Name	CAS Number	Chemical Name	Chemical Formula	Molecular weight (g/mol)	Smiles String
CGA 142856		[1,2,4]Triazol-1-yl-acetic acid			
CGA 131013		2-Amino-3-[1,2,4]triazol-1-yl-propionic acid			

¹ Identifications were not confirmed.

Data obtained from Table 2, p. 29 of the study report.

D. SUPPLEMENTARY EXPERIMENT-RESULTS: None were reported.

III. STUDY DEFICIENCIES

No significant deviations from good scientific practices or Subdivision N guidelines were noted in this study.

IV. REVIEWER'S COMMENTS

Mean results and standard deviations presented in this review were determined by the primary reviewer using Microsoft Excel 2000 (9.0.2720) software (DER Attachment 2). Standard deviations were determined using the "biased" or "n" method which determines the standard deviation of the entire sample population. The mean results for applied radioactivity reported by the study author were verified by the primary reviewer and there was consistent agreement (within \pm 0.1% of applied) between the study author's reported mean values and those determined by the primary reviewer (Table 4, p. 31; DER Attachment 2). For parent

PMRA Submission Number {.....}

EPA MRID Number 45930701

[¹⁴C]triazole and transformation products results, the study author only provided the mean result and not individual replicate results; consequently, those mean results could not be verified.

V. REFERENCES

- 1. U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Section 162-2, Anaerobic Soil Metabolism Studies. Office of Pesticide and Toxic Substances, Washington, DC. EPA 540/9-82-021.
- 2. U.S. Environmental Protection Agency. 1989. FIFRA Accelerated Reregistration, Phase 3 Technical Guidance. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 540/09-90-078.
- 3. U.S. Environmental Protection Agency. 1993. Pesticide Registration Rejection Rate Analysis Environmental Fate. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 738-R-93-010.
- 4. Wolfe, N., *et al.* 1990. Abiotic transformations in water, sediments and soil. *In* <u>Pesticides in the Soil Environment</u>, Soil Science Society of America, pp. 103-110.

Data Evaluation Report on the anaerobic biotransformation of 1,2,4-triazole (CGA 71019) in soil			
PMRA Submission Number {}	EPA MRID Number 45930701		
Attachment 1: Structures of Parent Comp	ound and Transformation Products		

PMRA Submission Number {.....} EPA MRID Number 45930701

4H-[1,2,4]Triazole [CGA 71019; R0; P]

IUPAC Name:

4H-[1,2,4]Triazole.

CAS Name:

Not reported.

CAS Number:

288-88-0.

SMILES String: n1cnnc1 (ISIS v2.3/Universal SMILES).

n1ncnc1 (EPI Suite, v3.12).

Unlabeled

$$\langle \rangle$$

[3,5-¹⁴C]-4H-[1,2,4]Triazole

* = Position of radiolabel.

Data Evaluation	Report on the	anaerobic	biotransformatio	n of 1,2,4-triazole	(CGA	71019)
in soil						

PMRA Submission Number {.....} EPA MRID Number 45930701

Identified Compounds

PMRA Submission Number {.....}

EPA MRID Number 45930701

4H-[1,2,4]Triazole [CGA 71019; R0; P]

IUPAC Name:

4H-[1,2,4]Triazole.

CAS Name:

Not reported.

CAS Number:

288-88-0.

SMILES String: n1cnnc1 (ISIS v2.3/Universal SMILES).

n1ncnc1 (EPI Suite, v3.12).

$$\langle \rangle$$

CGA 131013 [R1]

IUPAC Name:

2-Amino-3-[1,2,4]triazol-1-yl-propionic acid.

CAS Name:

Not reported.

CAS Number:

Not reported.

PMRA Submission Number {.....}

EPA MRID Number 45930701

CGA 142856 [R2]

IUPAC Name:

[1,2,4]Triazol-1-yl-acetic acid.

CAS Name:

Not reported.

CAS Number:

Not reported.

Carbon Dioxide

IUPAC Name:

Not reported.

CAS Name:

Not reported.

CAS Number:

Not reported.



ormation of 1,2,4-triazole (CGA 71019)
EPA MRID Number 45930701

Unidentified Reference Compounds

PMRA Submission Number {.....}

EPA MRID Number 45930701

NOA 457654 [R3]

IUPAC Name:

4H-[1,2,4]Triazol-3-ol.

CAS Name:

Not reported.

CAS Number:

Not reported.

Attachment 2: Excel and SigmaPlot Spreadsheets

PC Code: 600074 MRID: 45930701 Guideline: 162-2

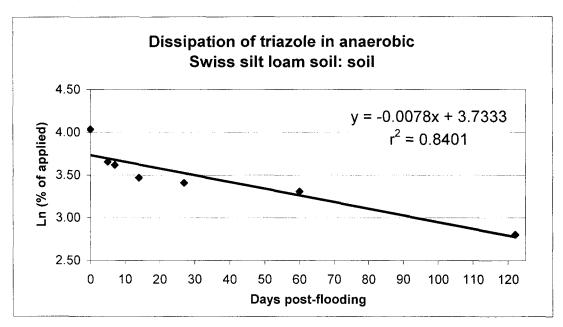
Anaerobic metabolism of [3,5¹⁴C]-labeled 4H-[1,2,4]-triazole in Swiss silt loam soil.

Triazole in soil

Half-life (days) 88.4 (0- to 122-day data)

	Triazole			
Days Post-flooding	(% of Applied)	Ln (% applied)		
0	56.5	4.034240638		
5	38.6	3.653252276		
7	37.2	3.616308761		
14	32.1	3.46885603		
27	30.2	3.407841924		
60	27.3	3.306886702		
122	16.5	2.803360381		

Results imported from Table 5, p. 32 of the study report.



PC code: 600074 MRID: 45930701 Guideline: 162-2

Anaerobic metabolism of [3,5¹⁴C]-labeled 4H-[1,2,4]-triazole in Swiss silt loam soil.

2.197224577

1.526056303

Triazole in water

(5- to 122-day data) 54.8 Half-life (days) Triazole (% of Applied) Ln (% applied) Days Post-flooding 20.0 2.995732274 22.4 3.109060959 2.708050201 14 15.0 2.610069793 27 13.6

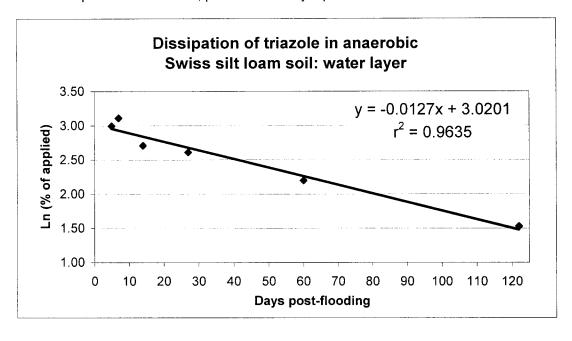
9.0

4.6

Results imported from Table 5, p. 32 of the study report.

60

122



PC Code: 600074 MRID: 45930701 Guideline: 162-2

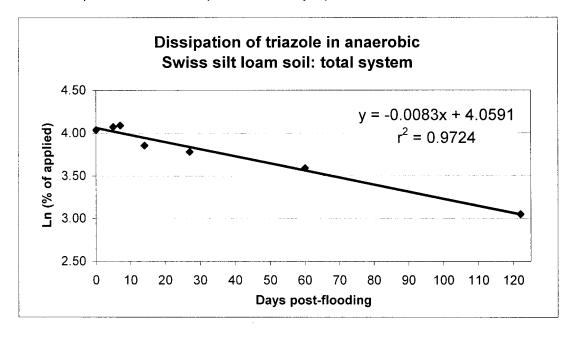
Anaerobic metabolism of [3,5¹⁴C]-labeled 4H-[1,2,4]-triazole in Swiss silt loam soil.

Triazole in total system

Half-life (days) 83.6 (0- to 122-day data)

	Triazole		
Days Post-flooding	(% of Applied)	Ln (% applied)	
0	56.5	4.034240638	
5	58.6	4.070734697	
7	59.6	4.087655574	
14	47.2	3.854393893	
27	43.8	3.779633817	
60	36.2	3.589059119	
122	21.1	3.04927304	

Results imported from Table 5, p. 32 of the study report.



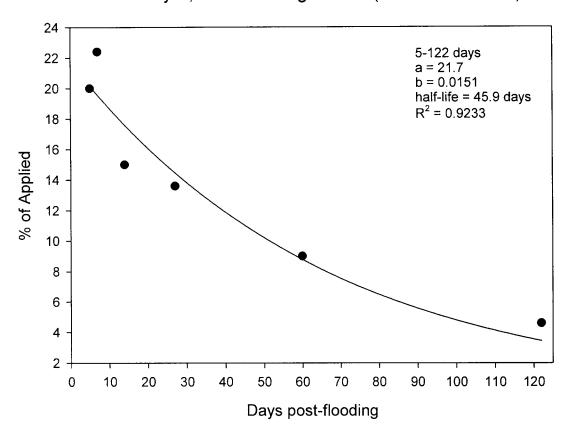
PC Code: 600074 MRID: 45930701 Guideline: 162-2

Nonlinear half-lives (exponential decay/single, 2 parameter)

Swiss silt loam soil

Phase	water	soil	system
Half-life (days)	45.9	72.2	81.5
R squared	0.9233	0.7218	0.9479

Triazole in anaerobic Swiss silt loam soil: water layer, nonlinear regression (MRID 45930701)



```
Chemical: Triazole
```

PC: 600074 MRID 45930701 Guideline: 162-2 Water layer

Nonlinear Regression

[Variables]

x = col(1)

y = col(2)

 $reciprocal_y = 1/abs(y)$

reciprocal_ysquare = $1/y^2$

'Automatic Initial Parameter Estimate Functions

xnear0(q) = max(abs(q))-abs(q)

yatxnear0(q,r) = xatymax(q,xnear0(r))

[Parameters]

a = yatxnear0(y,x) "Auto {{previous: 21.7445}}

b = if(x50(x,y)-min(x)=0, 1, -ln(.5)/(x50(x,y)-min(x))) "Auto {{previous: 0.0151114}}

[Equation]

f = a*exp(-b*x)

fit f to y

"fit f to y with weight reciprocal y

"fit f to y with weight reciprocal ysquare

[Constraints]

b>0

[Options]

tolerance=0.0001

stepsize=100

iterations=100

R = 0.96086184 Rsqr = 0.92325547

Adj Rsqr = 0.90406934

Standard Error of Estimate = 2.0593

	Coefficier	it Std. Error	t	P
a	21.7445	1.6046	13.5512	0.0002
b	0.0151	0.0033	4.5761	0.0102

Analysis of Variance:

•	DF	SS	MS	F	P
Regression	ı 1	204.0579	204.0579	48.1210	0.0023
Residual	4	16.9621	4.2405		
Total	5	221.0200	44.2040		

PRESS = 34.1365

Durbin-Watson Statistic = 2.5720

Normality Test: K-S Statistic = 0.1534 Significance Level = 0.9971

Constant Variance Test: Passed (P = 0.0600)

Power of performed test with alpha = 0.0500: 0.9236

Chemical: Triazole

PC:

MRID 45930701 Guideline: 162-2 Water layer
Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	20.1621	-0.1621	-0.0787	-0.1010	-0.0876
2	19.5619	2.8381	1.3782	1.6900	2.7368
3	17.5983	-2.5983	-1.2618	-1.4308	-1.7734
4	14.4596	-0.8596	-0.4174	-0.4753	-0.4238
5	8.7818	0.2182	0.1060	0.1443	0.1253
6	3.4410	1.1590	0.5628	0.7039	0.6513

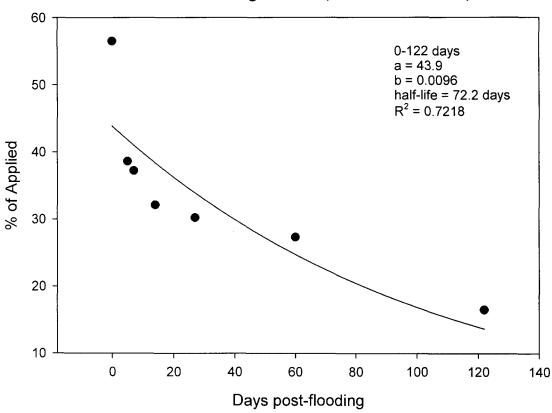
Influence Diagnostics:

Row	Cook'sDist	Leverage	DFFITS
1	0.0033	0.3929	-0.0705
2	0.7191	0.3349	1.9421
3	0.2926	0.2223	-0.9482
4	0.0335	0.2288	-0.2308
5	0.0089	0.4604	0.1157
6	0.1398	0.3607	0.4892

95% Confidence:

Row	Predicte	ed Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	20.1621	16.5785	23.7457	13.4145	26.9098
2	19.5619	16.2531	22.8707	12.9561	26.1677
3	17.5983	14.9026	20.2941	11.2773	23.9194
4	14.4596	11.7248	17.1943	8.1218	20.7973
5	8.7818	4.9024	12.6612	1.8725	15.6911
6	3.4410	0.0072	6.8749	-3.2283	10.1104

Triazole in anaerobic Swiss silt loam soil: soil, nonlinear regression (MRID 45930701)



Chemical: Triazole

PC: 600074 MRID 45930701 Guideline: 162-2

Soil

Nonlinear Regression

```
[Variables]
```

x = col(1)

y = col(2)

 $reciprocal_y = 1/abs(y)$

reciprocal_ysquare = $1/y^2$

'Automatic Initial Parameter Estimate Functions

xnear0(q) = max(abs(q))-abs(q)

yatxnear0(q,r) = xatymax(q,xnear0(r))

[Parameters]

a = yatxnear0(y,x) "Auto {{previous: 43.8649}}

b = if(x50(x,y)-min(x)=0, 1, -ln(.5)/(x50(x,y)-min(x))) "Auto {{previous: 0.00956102}}

[Equation]

f = a*exp(-b*x)

fit f to y

"fit f to y with weight reciprocal_y

"fit f to y with weight reciprocal_ysquare

[Constraints]

b>0

[Options]

tolerance=0.0001

stepsize=100

iterations=100

R = 0.84961241 Rsqr = 0.72184125

Adj Rsqr = 0.66620950

Standard Error of Estimate = 7.1027

	Coefficien	t Std. Error	t	P
a	43.8649	4.0286	10.8883	0.0001
b	0.0096	0.0035	2.7630	0.0397

Analysis of Variance:

,	DF	SS	MS	F	P
Regression	n 1	654.5780	654.5780	12.9753	0.0155
Residual	5	252.2391	50.4478		
Total	6	906.8171	151.1362		

PRESS = 516.7415

Durbin-Watson Statistic = 1.2039

Normality Test: K-S Statistic = 0.2616 Significance Level = 0.6534

Constant Variance Test: Passed (P = 0.0545)

Power of performed test with alpha = 0.0500: 0.7087

The power of the performed test (0.7087) is below the desired power of 0.8000. You should interpret the negative findings cautiously.

Chemical: Triazole

PC:

MRID 45930701 Guideline: 162-2

Soil

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	43.8649	12.6351	1.7789	2.1600	7.4703
2	41.8172	-3.2172	-0.4530	-0.5198	-0.4780
3	41.0252	-3.8252	-0.5386	-0.6087	-0.5658
4	38.3694	-6.2694	-0.8827	-0.9678	-0.9602
5	33.8848	-3.6848	-0.5188	-0.5696	-0.5268
6	24.7160	2.5840	0.3638	0.4554	0.4160
7	13.6627	2.8373	0.3995	0.5768	0.5340

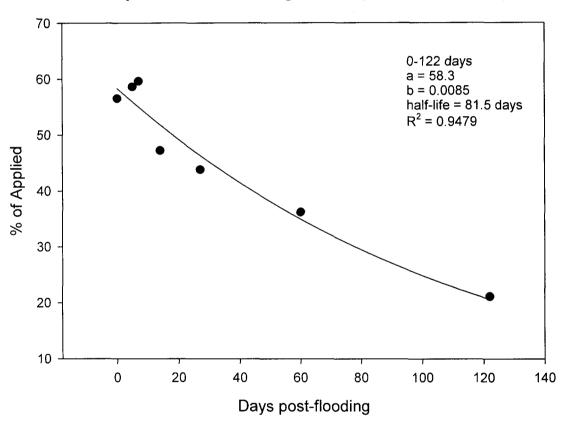
Influence Diagnostics:

Row	Cook'sD	ist Leverage	DFFITS
1	1.1065	0.3217	5.1448
2	0.0428	0.2405	-0.2690
3	0.0514	0.2172	-0.2980
4	0.0946	0.1681	-0.4316
5	0.0333	0.1703	-0.2387
6	0.0588	0.3617	0.3132
7	0.1806	0.5204	0.5563

95% Confidence:

Row	Predicte	ed Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	43.8649	33.5089	54.2208	22.8744	64.8553
2	41.8172	32.8630	50.7715	21.4817	62.1527
3	41.0252	32.5171	49.5334	20.8822	61.1683
4	38.3694	30.8832	45.8555	18.6363	58.1025
5	33.8848	26.3497	41.4199	14.1331	53.6365
6	24.7160	13.7349	35.6972	3.4102	46.0219
7	13.6627	0.4912	26.8342	-8.8505	36.1759

Triazole in anaerobic Swiss silt loam soil: total system, nonlinear regression (MRID 45930701)



Chemical: Triazole

PC: 600074 MRID 45930701 Guideline: 162-2 Total system

Nonlinear Regression

[Variables]

x = col(1)

y = col(2)

 $reciprocal_y = 1/abs(y)$

reciprocal ysquare = $1/y^2$

'Automatic Initial Parameter Estimate Functions

xnear0(q) = max(abs(q))-abs(q)

yatxnear0(q,r) = xatymax(q,xnear0(r))

[Parameters]

a = yatxnear0(y,x) "Auto {{previous: 58.3389}}

b = if(x50(x,y)-min(x)=0, 1, -ln(.5)/(x50(x,y)-min(x))) "Auto {{previous: 0.0085396}}

[Equation]

f = a*exp(-b*x)

fit f to y

"fit f to y with weight reciprocal_y

"fit f to y with weight reciprocal_ysquare

[Constraints]

b>0

[Options]

tolerance=0.0001

stepsize=100

iterations=100

R = 0.97358790 Rsqr = 0.94787340

Adj Rsqr = 0.93744808

Standard Error of Estimate = 3,5017

	Coefficie	ent Std. Error	t	P
a	58.3389	1.9528	29.8738	< 0.0001
b	0.0085	0.0012	7.2785	0.0008

Analysis of Variance:

Alluly 313 Of	v ai iaii				
	DF	SS	MS	F	P
Regression	1	1114.8481	1114.8481	90.9203	0.0002
Residual	5	61.3091	12.2618		
Total	6	1176.1571	196.0262		

PRESS = 99.0799

Durbin-Watson Statistic = 2.0910

Normality Test: K-S Statistic = 0.1460 Significance Level = 0.9960

Constant Variance Test: Passed (P = 0.1815)

Power of performed test with alpha = 0.0500: 0.9907

Chemical: Triazole

PC:

MRID 45930701 Guideline: 162-2 Total system

Total systemRegression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	58.3389	-1.8389	-0.5252	-0.6327	-0.5900
2	55.9004	2.6996	0.7709	0.8832	0.8598
3	54.9538	4.6462	1.3269	1.4989	1.8066
4	51.7651	-4.5651	-1.3037	-1.4297	-1.6632
5	46.3259	-2.5259	-0.7213	-0.7883	-0.7535
6	34.9491	1.2509	0.3572	0.4401	0.4015
7	20.5824	0.5176	0.1478	0.2233	0.2007

Influence Diagnostics:

Row	Cook's D	ist Leverage	DFFITS
1	0.0903	0.3110	-0.3964
2	0.1218	0.2380	0.4805
3	0.3102	0.2164	0.9493
4	0.2072	0.1686	-0.7489
5	0.0604	0.1628	-0.3323
6	0.0502	0.3413	0.2890
7	0.0320	0.5619	0.2274

95% Confidence:

, , , , , ,					
Row	Predicted	d Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	58.3389	53.3190	63.3589	48.0324	68.6455
2	55.9004	51.5090	60.2918	45.8850	65.9158
3	54.9538	50.7668	59.1408	45.0263	64.8813
4	51.7651	48.0695	55.4607	42.0346	61.4955
5	46.3259	42.6940	49.9577	36.6194	56.0323
6	34.9491	29.6903	40.2080	24.5242	45.3741
7	20.5824	13.8348	27.3301	9.3327	31.8321

PC Code: 600074 MRID: 45930701 Guideline: 162-2

Anaerobic metabolism of [3,5¹⁴C]-labeled 4H-[1,2,4]-triazole in Swiss silt loam soil. Determination of overall mean recovery of radioactivity.

					Soil											Study Reported		rted			
		Water		E	extracts		Non	extract	able		CO2		Vola	tile org	anics	Mate	erial Bal	lance	Mate	erial Ba	lance
Day	% AR	Mean	s.d.	% AR	Mean	s.d.	% AR	Mean	s.d.	% AR	Mean	s.d.	% AR	Mean	s.d.	% AR	Mean	s.d.	% AR	Mean	s.d.
O				90.5			5.6									96.1			96.2		
		######	######	91.9	91.2	0.7	5.0	5.3	0.3		#####	#####		######	#####	96.9	96.5	0.4	96.9	96.6	0.3
3													1								•
		######	#####	76.2			17.1			0.6				#####	#####	93.9			93.9		
4				75.5		1	19.2			1.3						96.0			96.1		}
		######	#####	79.6	77.6	2.1	15.8	17.5	1.7		1.0	0,3		#####	######	96.1	96.1	0.0		96.1	0.0
9	32.4			50.0			13.1			1.3						96.8			96.9		ĺ
I	28.8	30.6	1.8		52.0	2.0	12.0	12.6	0.5		1.3	0.0		#####	#####	96.1	96.5	0.4		96.5	0.4
11	33.1			49.9			12.3			1.2						96.5			96.5		
	34.6	33.9	0.8		50.3	0.3		11.6	0.8		1.2	0.1		#####	#####	97.1	96.8	0.3		96.9	0.4
18	30.2			49.5			15.0			1.0	,		:			95.7			95.7		
	29.8	30.0	0.2	46.4	48.0	1.5	18.5	16.8	1.8	0.4	0.7	0.3		######	#####	95.1	95.4	0.3	95.2	95.5	0.3
31	30.0			49.7			16.2			1.3				 ######	#####	97.2			97.2		
64	28.7		— <u> </u>	46.8			20.9			0.8			_	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	"""""	97.2			97.3		
	31.7	30.2	1.5		44.0	2.8	1	21.4	0.5	1.1	1.0	0.2		#####	######	95.8	96.5	0.7		96.6	0.8
126	36.6			42.2			17.2			1.3			 			97.3	- 33.0		97.3	34.4	
	36.6	36.6	0.0	40.2	41.2	1.0		16.4	0.8	3.9	2.6	1.3		#####	######	96.2	96.8	0.5	II .	96.7	0.6
Results	from Ta	able 4, p	o. 31 of t	he study	y report.										Overall	:	96.3	0.9		96.3	0.9

Means and standard deviations calculated using Microsoft program functions @average(A1:A2) and stdevp(A1:A2).

PC Code: 600074 MRID: 45930701 Guideline: 162-2

Anaerobic metabolism of [3,5¹⁴C]-labeled 4H-[1,2,4]-triazole in Swiss silt loam soil. Total [¹⁴C]residues in soil.

Total Ciresidues in soil.												
	Soil											
	Ext.	Nonext.	Total in Soil									
Day	% AR	% AR	% AR	Mean	s.d.							
0	90.5	5.6	96.1									
	91.9	5.0	96.9	96.5	0.4							
3												
	76.2	17.1	93.3	93.3	0.0							
4	75.5		94.7	•								
	79.6	15.8	95.4	95.1	0.4							
9	50.0	13.1	63.1									
	54.0	12.0	66.0	64.6	1.5							
11	49.9	12.3	62.2									
	50.6		61.4	61.8	0.4							
18	49.5	1	64.5									
	46.4	18.5	64.9	64.7	0.2							
31												
	49.7		65.9	65.9	0.0							
64	46.8	1	67.7									
	41.1			65.4	2.4							
126	42.2		59.4									
	40.2	15.5	55.7	57.6	1.8							

[14C]Residue water phase:soil ratios.

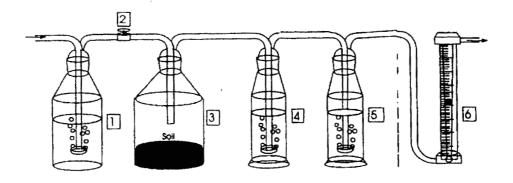
[,,,,,,,,	Water	Soil	Ratio	Ratio	W:S	ratio	S:W	S:W ratio		
Day	% AR	% AR	W:S	S:W	Mean	s.d.	Mean	s.d.		
0		96.1					· ·			
		96.9								
3										
		93.3								
4		94.7								
		95.4								
9		63.1	1	2						
	28.8	66.0		2	0	0	2	0		
11	33.1	62.2		2						
	34.6			2	1	0	2	<u> </u>		
18	30.2		l i	2				١ ,		
	29.8	64.9	0	2	0	0	2	0		
31				_	,	,	2	_ ا		
	30.0			2		0		0		
64			11	2	ll .		,	۱ ،		
	31.7			2	4	0	2	 		
126			41	2	И .] _	∥ ຸ			
	36.6	55.7	1	2	1	0	2	1 -		

Results imported from Mat bal worksheet.

Means and standard deviations calculated using Microsoft program functions @average(A1:A2) and stdevp(A1:A2).

Attachment 3: Illustration of Test System Flow-chart of Sample Work-up

Figure 1: Soil Metabolism Apparatus.



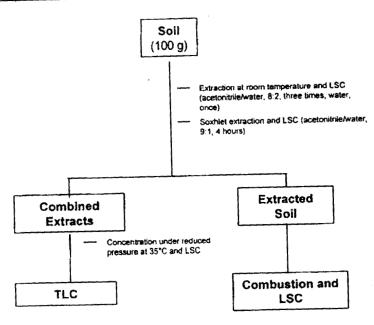
- 1) Water trap to moisten incoming air.
- 2) Valve to adjust the air-flow.
- 3) Metabolism flask containing 100g (dry weight)
- 4) NaOH trap
- 5) Ethylene glycol trap.
- 6) Flow-meter to check the air-flow (temporarily installed).

Note:

For establishment and maintaining anaerobic incubation conditions after 4 days of aerobic incubation, samples of soil were flooded with about 200 ml of oxygen-free, distilled water. Furthermore the continuous air-flow was replaced by purging with nitrogen four times a day for about 10 minutes.

Figure 2: Flow-chart of Sample Work-up.

Aerobic Incubation



Anaerobic Incubation

